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THE ATTENUATION OF HOG CHOLERA VIRUS AND ITS EFFECT ON NORMAL HOGS

CLIFFORD L. McARTHUR

From the Department of Bacteriology and Pathology of the Arkansas Experiment Station, Fayetteville

The discovery by Dorset, Bolton and McBryde¹ that hog cholera is caused by a filtrable virus, marks the beginning of a new era in the investigation of this disease. Following this, the discovery of the Dorset-Niles method of preparing an antiserum for the prevention of hog cholera was another important step in advance. Recently investigators have devoted their studies to methods whereby the filtrable virus may be attenuated and used as a vaccine. In each case, as the review of the literature shows, the results have been much the same, namely, a failure to attenuate the virus of hog cholera with any degree of accuracy or success.

HISTORICAL

Boxmeyer, McClintock and Seffer² found that drying hog cholera virus at 37 C. or adding one-third its weight in glycerol did not attenuate the virus. Graham³ states that virus heated at 60 C. for one hour did not produce sufficient immunity to protect hogs from cholera. He notes, also, that different strains of virus vary greatly. King⁴ reports that attempts to attenuate hog cholera virus by various physical and chemical means, so that it could be used as a vaccine, were not successful. Dorset and Niles⁵ were unsuccessful in their attempts to prepare a vaccine through an attenuated virus. Craig⁶ found that virus heated in a water bath at 51-60 C. did not confer any immunity and later work⁷ by the same author showed that he was not able to produce a successful vaccine. Lewis and others⁸ report some success in the attenuation of hog cholera virus by injecting the virus into a hyperimmune hog and allowing it to remain for varying lengths of time. Healy and Gott⁹ thought that possibly they had succeeded in attenuating hog cholera virus by mixing it with antiserum and incubating.

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¹ Bureau Animal Industry, Bull. 180.

² Jour. Infect. Dis., 1905, 2, p. 351.

³ Am. Vet. Rev., 41, p. 330.

⁴ Kans. Exp. Sta. Bull., 157, pp. 37-90.

⁵ U. S. Live Stock Sanitary Proceedings, 1911.

⁶ Ind. Sta. Report, 1912, p. 79.

⁷ Ibid., 1913, p. 76.

⁸ Okla. Sta. Bull., 104, 1914.

⁹ Jour. Infect. Dis., 1916, 19, p. 569.

EXPERIMENTAL WORK

In investigating the virus of hog cholera many obstacles are constantly met with, for as yet many of the phenomena connected with the disease are poorly understood. In most cases the disease is so closely associated with secondary invaders that they appear to be almost a part of the true infection, and their etiologic significance is still more or less a matter of inquiry. The lack of a specific test for hog cholera greatly handicaps the investigator and renders it more difficult to say just what does constitute a true cholera. The lack of a standard unit for measuring the virulence of hog cholera virus is another serious handicap. Reichel¹⁰ and later Robbins¹¹ have both devised means for maintaining a virulent strain of hog cholera virus over considerable periods of time, but as yet no definite unit has been established for measuring the virulence of the virus. Until some method is devised whereby the virus can be standardized, investigators must work to a large extent in the dark. Aside from the difficulties mentioned, there is the great variation by different hogs in their resistance to the disease and the great variation in virulence of different strains of virus.

The results given in this article are taken from a series of experiments on the attenuation of hog cholera virus by different methods, and on the effect of this treated virus when injected into healthy hogs, which had not been rendered immune to cholera by exposure or vaccination.

The experimental work differs from that of other investigators in the methods used for attenuation, the temperature and the quantity of heated material in each injection.

The virus was secured from different sources as will be noted in the different experiments. The work conducted by Dinwiddie¹² indicated that the serum-free corpuscles from hog cholera blood are more virulent than the plasma without the corpuscles from the same blood. In other words, that the causative organism is intracorpuseular. For this reason the blood used in these experiments was divided into two portions so that comparison could be made between the defibrinated blood and the blood corpuscles centrifuged as free as possible from serum. When the virus hog was killed one portion of the blood was collected in a sterile vessel, defibrinated and placed in tubes for heating. The other portion of the blood was collected in sterile salt solution 0.9%, filtered through gauze and the corpuscles washed as free as possible from serum by centrifuging nine times with sterile salt solution. In each case the quantity

¹⁰ Jour. Infect. Dis., 1913, 12, p. 39.

¹¹ Ibid., 1916, 19, p. 708.

¹² Ark. Exp. Sta. Bull., No. 111, 1912.

of virus required for each injection was placed in the bottom of tubes by means of a sterile pipet, care being taken that none of the virus touched the tube near the mouth. The heating was then conducted in a water bath, each tube being stoppered with cotton and immersed in the water to a much greater depth than the depth of the virus so that there would not be any chance for any of the virus to remain unheated.

The hogs used for testing the material were secured from the surrounding country. In no case were they taken from farms where cholera had been prevalent or where vaccination had been practiced. The hogs varied in weight from 40-60 lbs. and were of various breeds. After inoculation each hog was placed in a small shed with wood floor, these sheds being inclosed in larger pens. Each shed was carefully disinfected twice, one week apart, before using.

Exper. 1.—The attenuation of hog cholera virus and serum-free virus cells by heating at 55 C. for 2 hours.

The strain of virus in this experiment came from the Kentucky State Serum Plant and had been passed through two generations of hogs. The blood was collected from a virus hog which had been inoculated 6 days previously with 5 cc of this virus and was at the height of infection when killed.

In each part of the experiment 5 pigs, weighing from 40-60 lbs. were used.

Each animal was given 5 cc of the heated material, except the 2 check pigs which were each given 5 cc of the unheated material. Separate sterile syringes were used for each injection and the material was injected into the muscles of the ham. Ten days after the first injection each pig, except the check pigs, was given a second injection consisting of 10 cc of the heated material, the same strain of virus being used as in the first injection. Each pig was kept in a separate shed and all precautions taken against outside infection.

TABLE 1
INJECTION OF VIRUS HEATED AT 55 C. FOR TWO HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
50	5 cc of virus heated at 55 C. for 2 hours	10 cc of virus heated at 55 C. for 2 hours. Temperature of pig when injected 105.2 F.	January 21
51	5 cc of virus heated at 55 C. for 2 hours	10 cc of virus heated at 55 C. for 2 hours. Temperature of pig when injected 105 F.	January 28
52	5 cc of virus heated at 55 C. for 2 hours	10 cc of virus heated at 55 C. for 2 hours. Temperature of pig when injected 105.8 F.	January 23
53	5 cc of virus heated at 55 C. for 2 hours	10 cc of virus heated at 55 C. for 2 hours. Temperature of pig when injected 106 F.	January 20
54	5 cc of above virus unheated	January 17

We observe that both the virus and serum-free virus cells were quite virulent by the fact that check Pig 54 survived 9 days and 54E 7 days. The postmortem conditions were nearly alike, being that of acute cholera in both cases. None of the animals recovered from the disease; 21 days being the greatest length of time that any pig

survived. Pig 50A and the 2 check pigs did not live to receive the second injection. It was evident that heating the material attenuated it to a slight extent, as both of the check pigs succumbed several days earlier than the remaining pigs in the experiment. There was but little difference in the virulence of the heated material used in the two parts of the experiment as is shown in Tables 1 and 2. The serum-free corpuscles may have been slightly more virulent than the virus.

TABLE 2
INJECTION OF CORPUSCLES HEATED AT 55 C. FOR TWO HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
50A	5 c c of serum free corpuscles heated at 55 C. for 2 hours	January 18
51B	5 c c of serum free corpuscles heated at 55 C. for 2 hours	10 c c of serum-free corpuscles heated at 55 C. for 2 hours. Temperature of pig when injected 107.1 F.	January 20
52C	5 c c of serum free corpuscles heated at 55 C. for 2 hours	10 c c of serum-free corpuscles heated at 55 C. for 2 hours. Temperature of pig when injected 104.9 F.	January 29 chronic cholera
53D	5 c c of serum free corpuscles heated at 55 C. for 2 hours	10 c c of serum-free corpuscles heated at 55 C. for 2 hours. Temperature of pig when injected 105.3 F.	January 21
54E	5 c c of above cells unheated	January 15

TABLE 3
INJECTION OF VIRUS HEATED AT 55 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
55	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 104 F.	January 27
56	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 105.8 F.	January 23
57	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 105.8 F.	February 2
58	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 105.2 F.	January 24
59	5 c c of above virus unheated	January 16

Exper. 2.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 55 C. for 4 hours.

The strain of virus used in this experiment was the same as that in *Exper. 1* and had been passed through two generations since receipt. The blood was

collected from a virus pig which had been inoculated 8 days previously with 5 cc of this virus and was in the height of infection when killed.

Five pigs, weighing from 40-60 lbs. were used in each part of the experiment.

The same methods were followed and quantities used for inoculation as in Exper. 1.

TABLE 4
INJECTION OF CORPUSCLES HEATED AT 55 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
55A	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	10 cc of serum-free corpuscles heated at 55 C. for 4 hours. Temperature of pig when injected, 106.4 F.	January 27
56B	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	January 18
57C	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	10 cc of serum-free corpuscles heated at 55 C. for 4 hours. Temperature of pig when injected, 105 F.	January 23
58D	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	January 18
59E	5 cc of above corpuscles unheated	January 19

The material used in both parts of this experiment was virulent, as shown by the length of time that the check pigs survived, Pig. 59 living 8 days and 59E 11 days. The postmortem findings were those of acute cholera. None of the pigs recovered from the disease, 25 days being the greatest length of time that any pig survived.

In the second part of the experiment (Table 4) only 2 of the pigs lived to receive the second injection. Here again as in Exper. 1, heating seemed to attenuate the material, as the check pigs which received the unheated material, succumbed much earlier than the remaining pigs of the experiment. The serum-free corpuscles were possibly more virulent than the other material, but there was but little difference. The cases which lingered longest began to show slight symptoms of chronic cholera. The results indicate that there was but little difference in the virulence of the material when heated 4 hours and that heated 2 hours.

Exper. 3.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 60 C. for 4 hours.

The strain of virus used in this experiment was secured from the Arkansas State Serum Plant, 5 cc being injected into a pig weighing approximately 50 lbs. At the end of 8 days the pig was killed while in the height of infection and the blood collected for the experiment.

Five pigs weighing from 50-60 lbs. were used in each part of the experiment.

The same methods were followed and same quantities of material used for the inoculations as in Exper. 1. In this experiment the heated blood and serum-free corpuscles both coagulated. In order to make the injections it was necessary to add sufficient sterile salt solution to the coagulated material to place it in suspension.

TABLE 5
INJECTION OF VIRUS HEATED TO 60 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholc
60	5 cc of hog cholera virus heated at 60 C. for 4 hours	10 cc of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 102.4 F.	February 24
61	5 cc of hog cholera virus heated at 60 C. for 4 hours	10 cc of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 102 F.	February 26
62	5 cc of hog cholera virus heated at 60 C. for 4 hours	10 cc of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 102.6 F.	February 7
63	5 cc of hog cholera virus heated at 60 C. for 4 hours	10 cc of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 103.7 F.	January 21
64	5 cc of above virus unheated	January 17

TABLE 6
INJECTION OF CORPUSCLES HEATED TO 60 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
60A	5 cc of serum-free corpuscles heated at 60 C. for 4 hours	10 cc of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 102.8 F.	Feb. 20; chronic cholera
61B	5 cc of serum-free corpuscles heated at 60 C. for 4 hours	10 cc of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 103.3 F.	February 3
62C	5 cc of serum-free corpuscles heated at 60 C. for 4 hours	10 cc of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 101.8 F.	Feb. 27; chronic cholera
63D	5 cc of serum-free corpuscles heated at 60 C. for 4 hours	10 cc of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 103 F.	February 24 chronic cholera
64E	5 cc of above cells unheated	January 15

The virus and serum-free corpuscles were both quite virulent as shown by the length of time the 2 check pigs survived, Pig 64 living 9 days and 64E 7 days. Six of the remaining pigs survived a month or longer, and in each case the lesions found on necropsy varied from subacute to those of chronic cholera. The 2 remaining pigs lived 13 days and 26 days, respectively, and both showed marked lesions of hog cholera.

It was evident that heating the material attenuated it to some extent, as all animals except the check pigs lived to receive the second injection. The temperature of all pigs receiving the second injection was practically normal when the second injection was given, but outward symptoms would indicate that the animals had not entirely recovered from the first injection. There did not seem to be any difference in the virulence of the virus and serum-free virus cells after heating.

Exper. 4.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 65 C. for 2 hours.

The strain of virus used in this experiment was secured from the Arkansas State Serum Plant and had been passed through two generations of pigs since coming to the laboratory. The blood for this experiment was collected from a virus pig which had been inoculated 6 days previously with 5 cc of the virus and was in the height of infection when killed.

As in the previous experiments, 5 pigs weighing from 40-60 lbs. were used in each part of the experiment.

The same methods were followed and same quantities of material used for the inoculations as in *Exper. 1*. The coagulated material was placed in suspension in the same manner as in *Exper. 3*.

TABLE 7
INJECTION OF VIRUS HEATED AT 65 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Results
70	5 cc of hog cholera virus heated at 65 C. for 2 hours	10 cc of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 101 F.	Died April 25; chronic cholera
71	5 cc of hog cholera virus heated at 65 C. for 2 hours	10 cc of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 102 F.	Lived; given 5 cc of virus April 5
72	5 cc of hog cholera virus heated at 65 C. for 2 hours	10 cc of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 105.2 F.	Died April 27
73	5 cc of hog cholera virus heated at 65 C. for 2 hours	10 cc of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 101.8 F.	Died April 24
74	5 cc of above virus unheated	Died April 2

The virus and serum-free corpuscles were both quite virulent as was indicated by the length of time which the 2 check pigs survived, Pig 74 living 8 days and 74E 11 days. One pig survived all injections, although it was in a badly stunted condition when the experiment was closed. Three of the pigs lived a month or slightly longer, and all 3 showed marked lesions of chronic hog cholera on necropsy. One animal only lived 10 days and showed lesions of acute hog cholera.

The 2 remaining animals showed lesions of a subacute disease. All the pigs, except one, showed a normal temperature when the second injection was given.

There was but little difference in the virulence of this material, which was heated at 65 C. for 2 hours, and that in the previous experiment which was heated at 60 C. for 4 hours.

There did not seem to be any difference in the virulence of the virus and the serum-free corpuscles after heating.

TABLE 8
INJECTION OF CORPUSCLES HEATED AT 65 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Animal Died
70A	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	10 c c of serum-free corpuscles heated at 65 C. for 2 hours. Temperature of pig when injected, 103 F.	April 30 chronic cholera
71B	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	10 c c of serum-free corpuscles heated at 65 C. for 2 hours. Temperature of pig when injected, 102.5 F.	April 20 chronic cholera
72C	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	10 c c of serum-free corpuscles heated at 65 C. for 2 hours. Temperature of pig when injected, 102.1 F.	April 18 chronic cholera
73D	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	April 4
74E	5 c c of above serum-free corpuscles unheated	April 5

Exper. 5.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 70 C. for 2 hours.

This strain of virus was secured from the Michigan State Serum Plant and was used as soon as it reached the laboratory, 5 c c being injected into a pig of approximately 50 lbs. weight. The animal was killed on the 8th day and the blood collected for the experiment.

Five pigs weighing 50-60 lbs. were used in each part of the experiment.

The same methods were followed and the same quantities used for the inoculations as in *Exper. 1*. The coagulated material was placed in suspension in the same manner as in *Exper. 3*. After a month had passed 6 of the animals were in fair condition and each was given an injection of 5 c c of virulent virus to determine whether or not any immunity had been built up by the 2 previous injections.

The virus and serum-free corpuscles were both virulent as shown by the length of time that the 2 check pigs lived, Pig 84 surviving 9 days and 84E 10 days. The heated material showed much more evidence of attenuation than in previous experiments. While only one pig recovered, nearly all survived longer than in any of the previous

experiments. Three of the animals lived 2 months or longer. Six of the animals lived one month or longer and received a third injection, but only one pig had enough immunity to withstand the third injection. Pig 80A lived 20 days and showed only lesions of acute cholera on necropsy. All of the other animals, except the check pigs, showed marked lesions of chronic cholera.

TABLE 9
INJECTION OF VIRUS HEATED AT 70 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Results
80	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 102 F.	Died, May 14; chronic cholera
81	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 101.5 F.	Given 5 c c of strong virus on April 25. Died June 1; chronic cholera lesions on necropsy.
82	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 102.5 F.	Given 5 c c of strong virus on April 25. Died May 20; chronic cholera lesions on necropsy
83	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 102 F.	Given 5 c c of strong virus on April 25; lived
84	5 c c of above virus unheated	Died, April 3; acute cholera

TABLE 10
INJECTION TO CORPUSCLES HEATED AT 70 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Results
80A	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 103 F.	Died April 14; acute cholera
81B	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 102.2 F.	Given 5 c c of strong virus on April 25. Died May 3; chronic cholera
82C	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 102.6 F.	5 c c strong virus April 25. Died, May 27; chronic cholera
83D	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 101.8 F.	5 c c strong virus April 25. Died June 4; chronic cholera
84E	5 c c of above serum-free corpuscles unheated	Died April 4; acute cholera

From the length of time that the different animals survived it would appear that the material was attenuated by heating and some immunity built up by the injections, but not enough to withstand an injection of strong unheated virus.

Exper. 6.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 70 C. for 4 hours.

This was also a Michigan strain of virus and had been passed through four generations of pigs since coming to the laboratory. The blood used in this experiment was collected from a pig which had been inoculated 7 days previously with 5 cc of this virus and was in the height of infection when killed.

Five pigs averaging 40-60 lbs. were used in each part of the experiment.

This experiment differs considerably from the previous experiments, only $\frac{1}{10}$ of the quantity or 0.5 cc of the heated material being used for the inoculations. The check pigs each received 0.5 cc of the unheated material. At the end of 21 days each pig was given 1 cc strong unheated virus to determine whether or not any immunity had been built up by the first injection.

TABLE 11
INJECTION OF VIRUS HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
90	0.5 cc of hog cholera virus heated at 70 C. for 4 hours	1 cc of unheated virus	Recovered
91	0.5 cc of hog cholera virus heated at 70 C. for 4 hours	1 cc of unheated virus	Recovered
92	0.5 cc of hog cholera virus heated at 70 C. for 4 hours	1 cc of unheated virus	Recovered
93	0.5 cc of hog cholera virus heated at 70 C. for 4 hours	Too sick for second injection; died November 20; cholera lesions on necropsy
94	0.5 cc of virus, unheated	Died October 10; acute cholera

The length of time which the check pigs survived would indicate that the unheated material was fully virulent, check Pig 94 living 8 days and 94E 10 days after inoculation. The heated material showed a much greater attenuation than in any of the previous experiments. Five of the pigs withstood an injection of virulent hog cholera virus 21 days after the first injection of heated material, and finally recovered. They were badly stunted, however. The 3 remaining pigs lived a month or longer, but finally all developed chronic cholera. There was evidently some immunity produced by the first injection, but not enough to protect all of the animals from 1 cc of virulent virus.

TABLE 12
INJECTION OF CORPUSCLES HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
90A	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died, Nov. 3; chronic cholera
91B	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
92C	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died, Nov. 17; chronic cholera
93D	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
94E	0.5 c c of serum-free corpuscles unheated	Died, October 12; acute cholera

Exper. 7.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 70 C. for 4 hours.

The blood used in this experiment was the same as that used in the preceding experiment and was secured from the same virus pig.

Five pigs weighing from 40-50 lbs. were used in each part of the experiment.

Each animal was given 0.25 c c of the heated material, except the check pigs which were given 0.25 c c of the unheated material. After 21 days a second injection, consisting of 1 c c of virulent unheated virus, was given each animal except the check pigs. This virus was the same strain as that used in the first injection.

TABLE 13
INJECTION OF 0.25 C C OF VIRUS HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
95	0.25 c c of virus heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
96	0.25 c c of virus heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
97	0.25 c c of virus heated at 70 C. for 4 hours	Died October 11; acute cholera
98	0.25 c c of virus heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 8; chronic cholera
99	0.25 c c of above virus unheated	Died October 12; acute cholera

The material of this experiment was virulent, check Pig 99 surviving 10 days and 99E 7 days. Four of the pigs recovered from the inoculations, but one animal died in 9 days with acute cholera. The 3 remaining animals survived for a month or longer, finally developing cases of chronic cholera and had well marked chronic lesions. There did not seem to be any great difference in the results with 0.5 c c of

heated material and with 0.25 c c. There was a marked difference, however, in the results of these two experiments and the preceding ones in which larger quantities of heated material were used.

TABLE 14
INJECTION OF 0.25 c c CORPUSCLES HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
95A	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 26; chronic cholera
96B	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 11; chronic cholera
97C	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
98D	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 17; chronic cholera
99E	0.25 c c of serum-free corpuscles unheated	Died October 9; acute cholera

SUMMARY

The virus used in each of these experiments was fully virulent, until heated, as shown by the length of time (6-10 days) required to kill the check pigs. Heating at 55 C. for 2 hours attenuated the material to a slight extent as did also heating at 55 C. for 4 hours, at 60 C. for 4 hours, and at 65 C. for 2 hours. There was but little difference in effect on normal pigs of the material heated at these temperatures, the material in practically every case being virulent enough to cause hog cholera.

Material heated at 70 C. for 2 hours showed considerable evidence of attenuation when injected in quantities of 5 c c. When heated at 70 C. for 4 hours in quantities of 0.5 and 0.25 c c, greater evidence of attenuation was shown than in any of the other experiments, and in only one case, Fig. 97, was the material virulent enough to cause disease. In the remaining animals some immunity was produced from the first injection, although all did not withstand an injection of 1 c c of virulent unheated hog cholera virus 21 days later. Although a number of the animals recovered in these last two experiments they were in a badly stunted condition. In all experiments the animals inoculated with heated material soon developed a marked cachexia and finally died in an emaciated condition, in most cases showing typical

lesions of chronic hog cholera. Some pigs were more resistant to the heated material than others.

There was but little, if any, difference in the virulence of the virus and the serum-free corpuscles.

The results of these experiments would indicate that hog cholera virus can be attenuated to a certain extent by heating, but this heated material is not suitable as vaccine, because in many cases it is apt to cause the disease.